Report No. 39

Annual Summary Report | *October 1, 1961-September 30, 1962*

SYNTHESIS OF POTENTIAL ANTIRADIATION DRUGS

Prepared for:

DEPARTMENT OF NUCLEAR MEDICINE
WALTER REED ARMY INSTITUTE OF RESEARCH
WALTER REED ARMY MEDICAL CENTER
WASHINGTON 12, D.C.

CONTRACT NO. DA 49-193-MD-2068

STANFORD RESEARCH INSTITUTE

MENLO PARK, CALIFORNIA

*SRI

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Approved:

Bruce Graham

BRUCE GRAHAM, GENERAL MANAGER
LIFE SCIENCES RESEARCH

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A B S T R A C T

Stanford Research Institute

Synthesis of Potential Antiradiation Drugs

Principal Investigators: Leon Goodman
James E. Christensen

28 pages November 1, 1962

Contract No: DA-49-193-MD-2068

Supported by: U. S. Army Medical Research and Development Command
Department of the Army
Washington 25, D. C.

The work of this report is an extension of the research on the preparation of mercaptoamino sugars that was started in 1959. During this report period the trans-hexoside, methyl 2-amino-2,3-dideoxy-3-mercapto-D-altropyranoside hydrochloride was synthesized, employing the episulfonium ion approach and the two trans-pentosides, methyl 2-amino-2,3-dideoxy-3-mercapto-α-D-arabinofuranoside hydrochloride and methyl-3-amino-2,3-dideoxy-2-mercapto-α-D-xylofuranoside hydrochloride, were prepared by a similar method.

The cis-pentoside, methyl 3-amino-2,3-dideoxy-2-mercapto-α-D-ribofuranoside hydrochloride, was synthesized by the "complex neighboring group" method that has been employed successfully in the hexose series.

The "pendant" β-mercaptoethylamino sugars, methyl 3-(β-mercaptoethyl)-amino-3-deoxy-4,6-O-ethylidene-α-D-altropyranoside hydrochloride and methyl 3-(β-mercaptoethyl) amino-3-deoxy-D-altropyranoside hydrochloride, were prepared as examples of uniquely-substituted MEA molecules.

Significant progress was made toward the synthesis of methyl 6-amino-6-deoxy-L-idothiapyranose hydrochloride and its free sugar which represent the ring structures that form from 6-amino-5,6-dideoxy-6-mercapto-L-idose derivatives.

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SYNTHESIS OF POTENTIAL ANTIRADIATION DRUGS

I Introduction

The principal objective of the research is to attempt to synthesize antiradiation compounds that would be less toxic and more effective than those now available. Emphasis has been placed on the classes of compounds that contain mercaptan and amino (or substituted amino) groups, since such compounds can be considered as derivatives of β-mercaptoethylamine, a very effective antiradiation chemical.

II Summary

The year's work under Contract No. DA49-193-MD-2068 has been concerned with four areas of research:

1. synthesis of trans-β-mercaptoamino sugars
2. synthesis of cis-β-mercaptoamino sugars
3. preparation of sugars bearing pendant β-mercaptoethylamine groups
4. synthesis of 6-amino-6-deoxy-L-idothiapyranose hydrochloride.

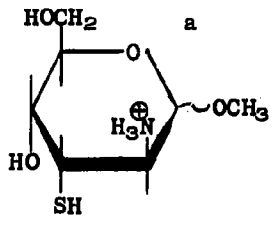
The work in area 1 resulted in the preparation of the hexose trans-mercaptoamino glycoside (VII), which is currently being evaluated. In the pentose series, the two trans-mercaptoamino glycosides (XVIII and XXI) were prepared on a small scale and submitted for microbiological testing; the large-scale preparation of XVIII and XXI, to furnish enough of the compounds for testing in mice, is in progress.

The "complex neighboring group" approach, used in the work of area 2, permitted the preparation of the pentose cis-mercaptoamino glycoside (XXXIII), which was prepared in a quantity sufficient for evaluation in mice. The pentose 3,5-mercaptoamine (XXXVII), an analog of 3-mercaptopropylamine, was also prepared from some of the intermediates used to prepare XXXIII.

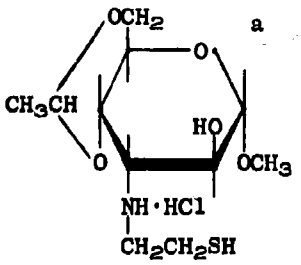
The blocked pendant mercaptoethylamino sugar (XXXIX) as well as its deblocked glycoside (XLI) were prepared in the work of area 3 and have been shown to be ineffective antiradiation compounds.

The work in area 4 is still in progress. The sequence of reaction steps has been carried through the preparation of the glycoside hydrochloride (XLIV) but, to date, the analytical data for this compound have not been satisfactory and further efforts are required to complete the synthesis.

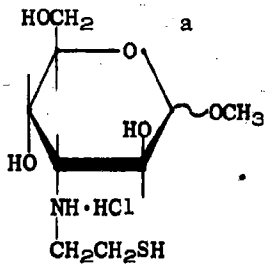
The following compounds were submitted to the sponsor for biological testing:



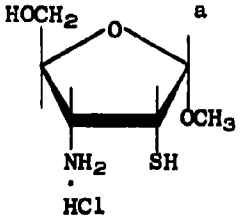
VII



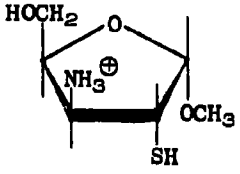
XXXIX



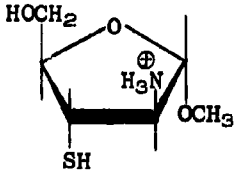
XLI



XXXIII



XXI



XVIII

Two manuscripts that cover a part of this work were accepted for publication:

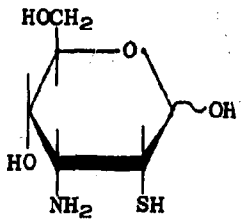
Preparation of a Glycoside of 2-Amino-2,3-dideoxy-3-mercapto-D-altrose. L. Goodman and J. E. Christensen. J. Org. Chem.

Preparation of Sugars and Carbohydrate-like Compounds Carrying a β-Mercaptoethylamine Moiety. J. E. Christensen and L. Goodman. J. Org. Chem.

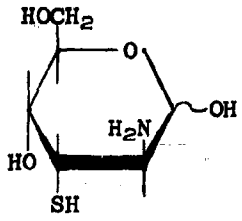
III Method of Approach

The synthesis of sugars that contain a β-mercaptoethylamine moiety has been the objective of the research to this date. The preparation of such compounds as A, B, and C is being sought to test the hypothesis that these types of sugars may retain the effective antiradiation qualities of,

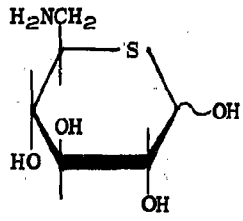
[a] Submitted in an amount sufficient for testing in mice.



A



B



C

but may have a lower toxicity than, β-mercaptoethylamine. The work of the year, then, may divided into four main parts:

A. synthesis of trans-β-mercaptoamino sugars
B. synthesis of cis-β-mercaptoamino sugars
C. preparation of sugars bearing pendant β-mercaptoethylamine groups
D. synthesis of 6-amino-6-deoxy-L-idothiapyranose hydrochloride.

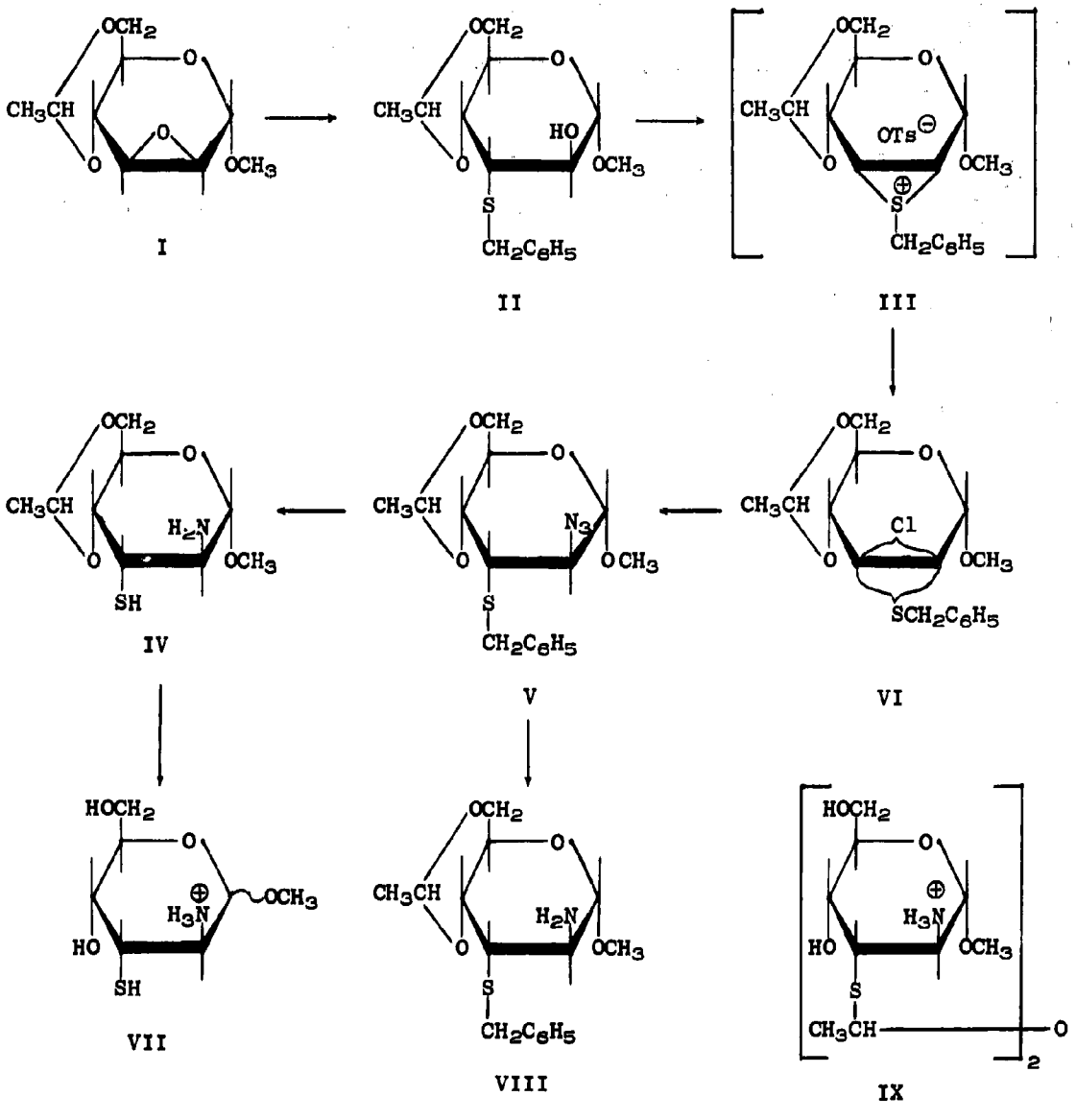
A. Synthesis of trans-β-Mercaptoamino Sugars

Report 26 described the preparation of methyl 2-amino-2,3-dideoxy-3-mercapto-D-altropyranoside (VII) by a very involved procedure, which required a change from the 4,6-O-benzylidene to the 4,6-O-ethylidene blocking group in the course of the synthesis. The development of a practical method of preparation of methyl 2,3-anhydro-4,6-O-ethylidene-α-D-mannopyranoside (I) (Report 26) provided the pathway to a much shorter synthesis of VII.

Ring opening of I with sodium benzyl mercaptide furnished an excellent yield of II, whose structure is assumed on the basis of trans-diaxial ring cleavage of I, according to the considerations elaborated in an earlier paper describing some similar studies.[1] Treatment of II with tosyl chloride in pyridine afforded either a mixture of chloride and tosylate (according to infrared evidence) or a pure, sirupy chloroglycoside (VI), depending on the length of time the reaction was allowed to proceed. The formation of the chloride (VI) can be rationalized by assuming the intervention of the episulfonium ion intermediate (III). The reaction of VI with sodium azide afforded an excellent yield of a crystalline, sharply melting azide (V) whose structure is written on the assumption of trans-diaxial opening at C.2 of an episulfonium ion intermediate corresponding to III.[1] The azide (V) was reduced to the crystalline amine (VIII) with sodium borohydride in refluxing isopropanol,[2] although this was not a necessary step in the preparation of VII. Treatment of V with sodium in liquid ammonia directly afforded the blocked mercaptoamine (IV) as a nitroprusside-positive

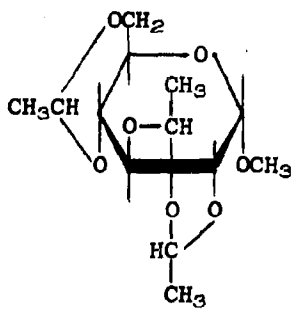
[1] J. E. Christensen and L. Goodman, J. Am. Chem. Soc. 83, 3827 (1961)

[2] P. A. S. Smith, J. H. Hall, and R. O. Kan, J. Am. Chem. Soc. 84, 485 (1962)



sirup. When IV was treated with methanolic hydrogen chloride either at room temperature or at reflux, hygroscopic solids were obtained whose n.m.r. spectra in deuterium oxide showed varying amounts of a doublet centered at $\tau = 8.36$. Since the doublet attributable to the O-ethylidene methyl group in V was found at $\tau = 8.62$, it was apparent that the secondary methyl group of the supposedly deblocked product from IV was not part of a simple 4,6-O-ethylidene group. Under the proper conditions, it was possible to isolate from treatment of IV with methanolic acid a material that gave good analytical agreement with structure IX. It seems probable that the proximity of

the C.4 hydroxyl group and the C.3 thiol group is reponsible for the formation of IX, since such a difficulty was not encountered in the deblocking step that afforded the isomeric methyl 3-amino-2,3-dideoxy-2-mercapto-D-altropyranoside (Report 13). Structure IX bears a relationship to methyl 4,6-O-ethylidene-2,3-oxidodiethylidene-α-D-glucoside (X), which is formed



X

when methyl α-D-glucoside is treated with paraldehyde and acid.[3] Attempts to complete the conversion of IV to VII by more vigorous acid treatment or by reaction with mercuric chloride were fruitless. When the deblocking of IV was conducted in the presence of excess ethanedithiol, however, the formation of IX was prevented and VII was isolated as a hygroscopic, amorphous solid that could be purified by precipitation with ether from a methanol solution. Similarly to the isomeric 3-amino-2-mercaptoaltroside (Report 13), it tenaciously retained ether, as evidenced both by elemental analysis and by the ether C-methyl n.m.r. triplet at τ = 8.78. Attempts to hydrolyze VII to the free sugar with aqueous hydrochloric acid were not encouraging; the reaction mixtures darkened rapidly.

Two pentose trans-β-mercaptoamines (XVIII and XXI) were synthesized by the same method used to prepare VII, starting with methyl 2,3-anhydro-α-D-lyxofuranoside (XI).[4] The epoxide (XI) was opened with sodium benzyl mercaptide to give an excellent yield of the 3-benzylthio glycoside (XII), characterized as the crystalline dibenzoate (XIII). Ring opening of XI was assumed to occur predominantly at C.3 since this is the situation that is generally observed with 2,3-anhydro derivatives of pentose furanosides.[5] The high yield of the crystalline, sharply melting dibenzoate (XIII) is confirming evidence that predominantly one isomer was formed in the ring opening of XI. Reaction of XII with slightly more than one equivalent of methyl chloroformate yielded XIV as a sirup, which was converted to the

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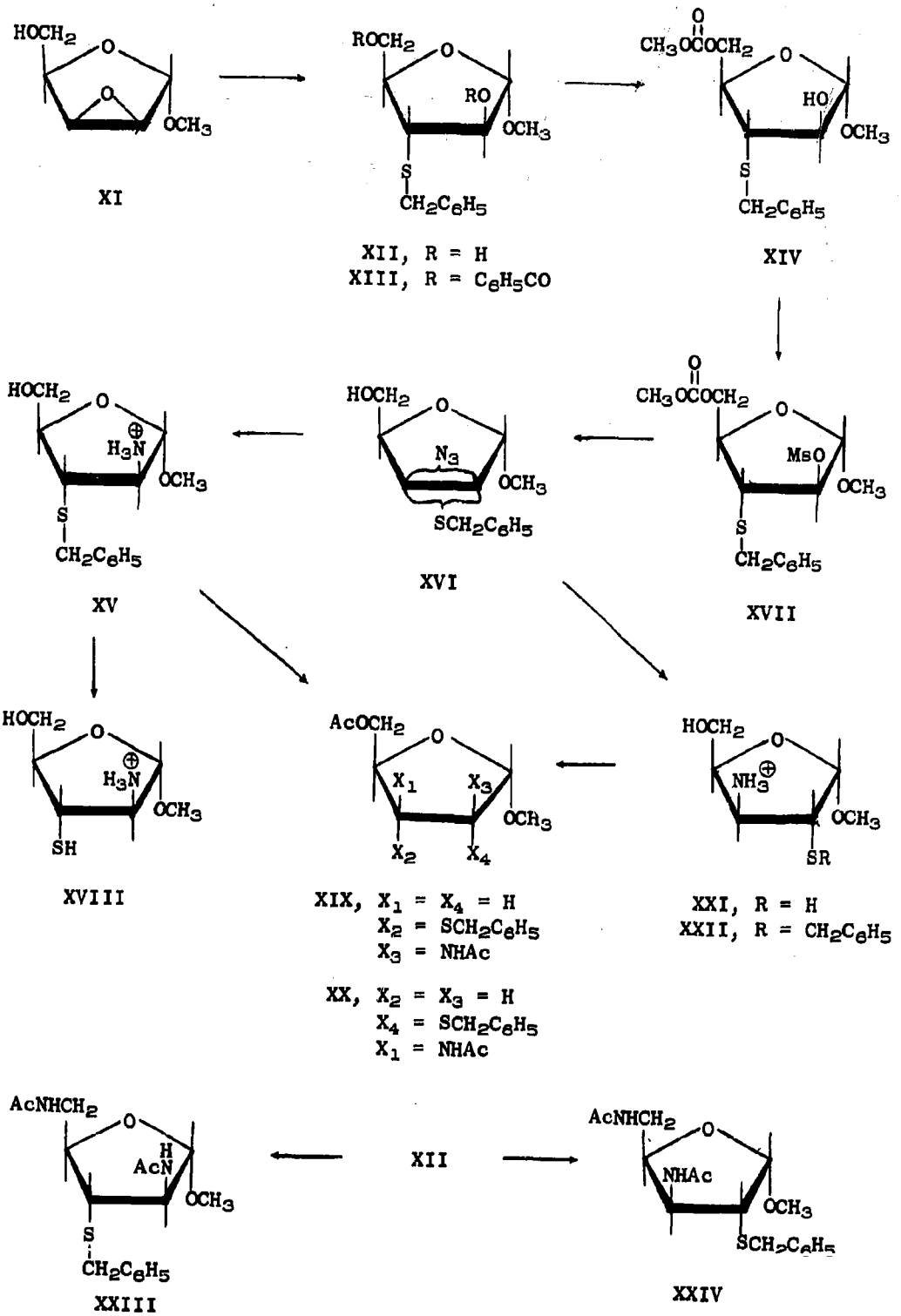
[3] H. Appel and W. N. Haworth, J. Chem. Soc. 793 (1938)

[4] B. R. Baker, R. E. Schaub, and J. H. Williams, J. Am. Chem. Soc. 77, 7 (1955)

[5] For leading references, see C. D. Anderson, L. Goodman, and B. R. Baker, J. Am. Chem. Soc. 80, 5247 (1958)


XI
XII, R = H
XIII, R = C6H5CO
XIV
XV
XVI
XVII
XVIII
XIX, X1 = X4 = H
X2 = SCH2C6H5
X3 = NHAc
XX, X2 = X3 = H
X4 = SCH2C6H5
X1 = NHAc
XXI, R = H
XXII, R = CH2C6H5
XXIII
XXIV

mesylate (XVII), also a sirup, with methanesulfonyl chloride in pyridine. Subsequent reactions that yielded XXIII and XXIV showed that the formation of XIV was not complete and that XVII contained some 2,5-di-O-mesylate.

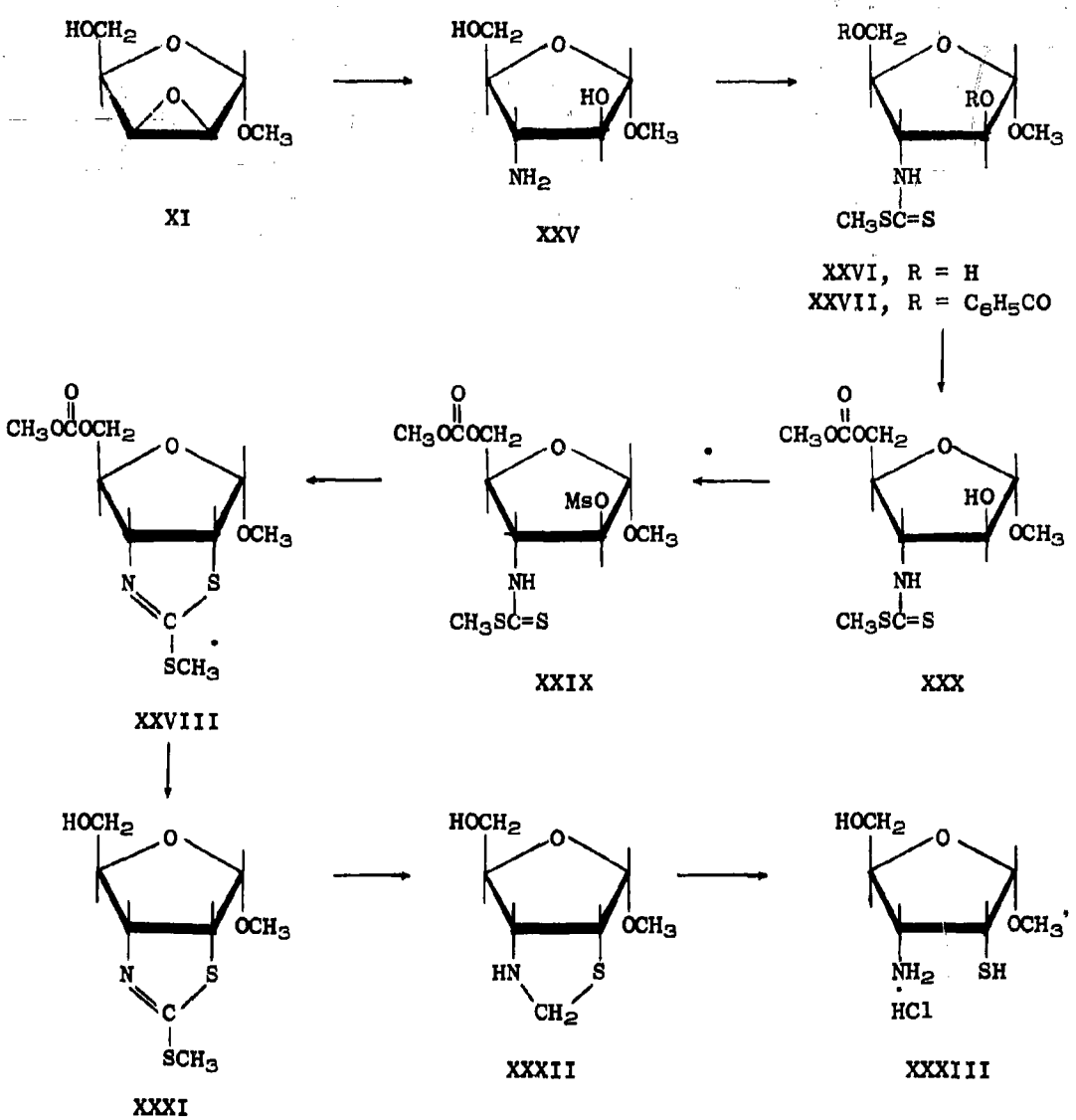
When the crude mesylate was treated with sodium azide in aqueous 2-methoxyethanol, the carbomethoxy group was eliminated and a mixture of azides (XVI) was formed. The azide mixture undoubtedly arose from the intermediate conversion of XVII to an episulfonium ion. The azide (XVI) was reduced with sodium borohydride in isopropanol[2] and the amine product was acetylated, giving a mixture of XIX and XX that contained some of the diamides (XXIII and XXIV) formed from the dimesylate contaminant of XVII. Chromatography on silicic acid served to separate the the mixture of the diamides (XXIII and XXIV) from XIX and XX. Saponification of the mixture of XIX and XX and conversion of the free amines to their hydrochlorides gave a mixture of XV and XXII which was easily separated into the crystalline components by recrystallization. The n.m.r. spectra of the N,O-diacetates (XIX and XX) that were derived from the hydrochlorides (XV and XXII, respectively) served to assign the correct structures to the hydrochlorides. Thus the C.1 proton of XIX, which is in a trans-relationship to the C.2 proton, appeared as a single peak and is therefore not detectably spin-coupled to the C.2 proton. The C.1 proton of XX, however, which is cis to the C.2 proton, showed a well resolved doublet with $J = 5$ cps as a result of the spin-coupling to the C.2 proton.

Reduction of the free amines of XV and XXII with sodium in liquid ammonia afforded the mercaptoamines, which were isolated as their mercaptides by precipitation with mercuric chloride. The mercaptide from XV was analyzed and on the basis of the elemental analyses appears to be the bis-mercury salt (XLVIII) (see Experimental). Regeneration of the mercury salts with hydrogen sulfide afforded the hydrochlorides (XVIII and XXI), which were isolated as analytically pure, but amorphous solids.

The mixture of N,N-diacetates (XXIII and XXIV) isolated by chromatography was separated by fractional crystallization. Again, n.m.r. spectroscopy served to assign structures to the compounds, since in XXIII the C.1 proton appeared as a singlet and in XXIV it appeared as a doublet, verifying the trans- and cis-relationship, respectively, of the C.1 and C.2 protons in these two diamides.

B. Synthesis of cis-β-Mercaptoamino Sugars

The "complex neighboring group" that had been used previously to prepare a cis-mercaptoamino hexoside (Report 26) was successfully employed in the preparation of the cis-mercaptoamino pentoside (XXXIII). The starting material for the sequence again was the anhydrolyxoside (XI). This was converted to the 3-aminoarabinoside (XXV) by use of the literature procedure.[4] The preparation of XXVI from XXV under the standard conditions for synthesis of these dithiocarbamates gave a crystalline product which was further characterized as the crystalline dibenzoate (XXVII). The reaction of XXVI with nearly the stoichiometric amount of methyl chloroformate

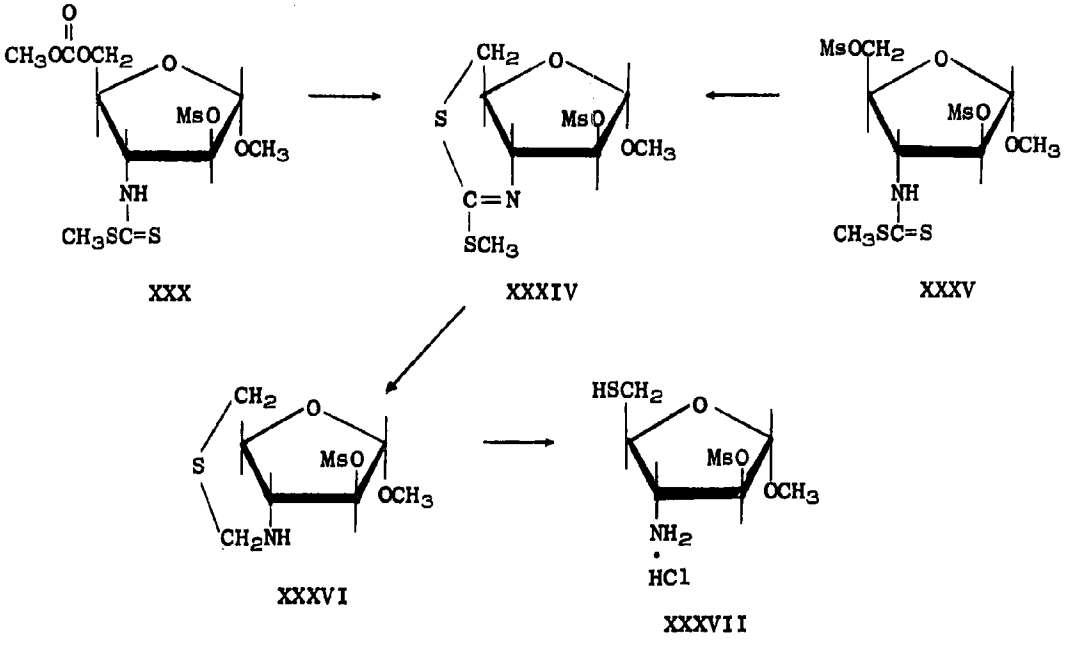


XI XXV XXVI, R = H XXVII, R = C_6H_5CO XXIX XXX XXVIII XXXI XXXII XXXIII

in pyridine yielded the 5-O-carbomethoxy derivative (XXX) as an oil; use of the trityl blocking group for the C.5 hydroxyl was less satisfactory. Mesylation of XXX afforded only a fair yield of the crystalline XXIX; there was infrared evidence for partial cyclization during the esterification of XXX. Several methods were investigated for the conversion of XXIX to the thiazoline (XXVIII). When XXIX was heated for a short time in refluxing toluene, the product appeared to be the methanesulfonic acid salt of XXVIII. It was more satisfactory to employ refluxing pyridine for the conversion of XXIX to XXVIII. The action of methanolic sodium methoxide on XXIX gave a second heterocycle (XXXIV) whose formation is discussed below.

The thiazoline (XXVIII), an oil, was deblocked with a catalytic amount of sodium methoxide to afford XXXI, also as an oil. Reduction of XXXI with aluminum amalgam afforded the thiazolidine (XXXII), which was an oil that could not be converted to a crystalline derivative. The standard decomposition of XXXII with mercuric chloride yielded a solid mercaptide that was converted to the desired mercaptoamine (XXXIII) with hydrogen sulfide. The product (XXXIII) was amorphous but gave excellent elemental analyses and was chromatographically homogeneous.

Treatment of XXX with methanolic sodium methoxide resulted in a low yield of a crystalline compound whose infrared spectrum showed a strong C=N thiazoline band at 6.4 μ and strong sulfonate ester bands. Since the

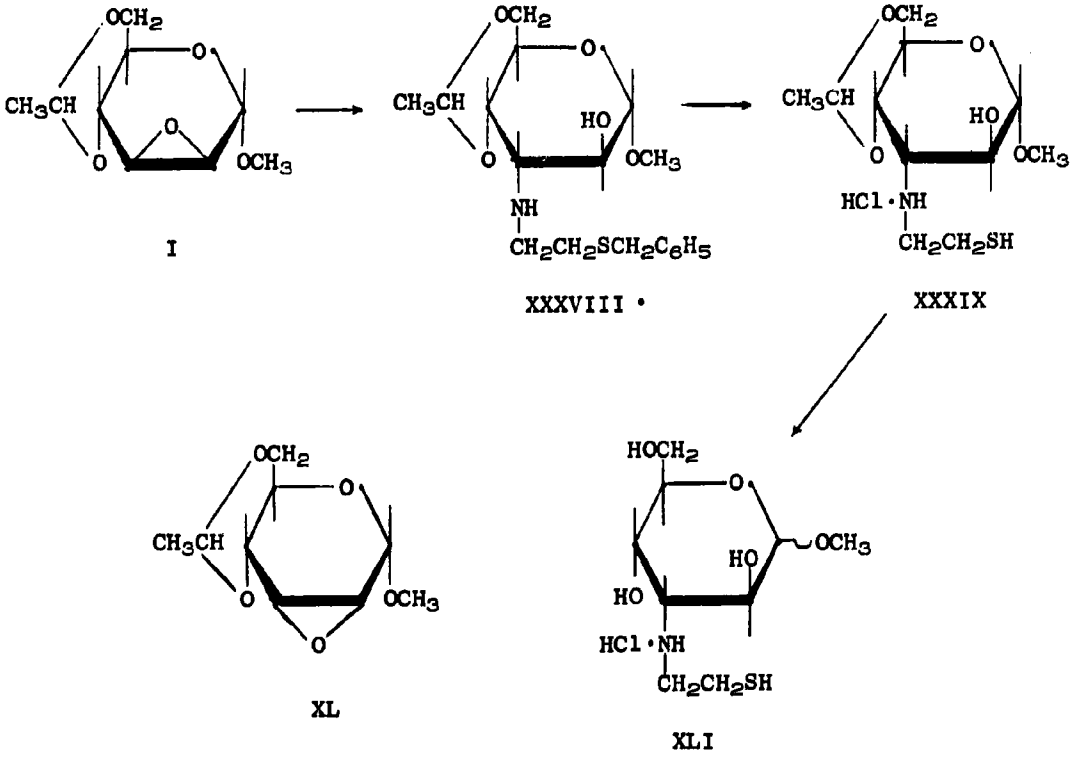


same compound could be obtained by sodium methoxide treatment of the dimesylate (XXXV) and the mesyl group in the new thiazoline(?) could not be displaced by benzoate ion under forcing conditions, it was clear that the new compound was the dihydro-1,3-thiazine (XXXIV). It seems probable that the first step in the conversion of XXX to XXXIV is the removal of the carbomethoxy blocking group of XXX. This is followed by a transmesylation reaction between deacylated XXX and another molecule of XXX to form XXXV, which then undergoes 3,5-cyclization to XXXIV. A similar intermolecular transtosylation reaction has been observed by Cope and Shen[6] so that there is precedent for such a step. Aluminum amalgam reduction of XXXIV afforded the crystalline tetrahydro-1,3-thiazine (XXXVI), which was converted to the mercaptoamine (XXXVII) with mercuric chloride, then hydrogen sulfide.

[6] A. C. Cope and T. Y. Shen, J. Am. Chem. Soc. 78, 5912 (1956)

C. Preparation of Sugars Bearing Pendant β-Mercaptoethylamine Groups

The mannose epoxide (I) was the starting material for preparation of the pendant β-mercaptoethylamino sugars (XXXIX and XLI). Ring opening of I with excess S-benzyl-β-mercaptoethylamine (BzMEA)[7] gave the blocked



I

XXXVIII

XXXIX

XL

XLI

derivative (XXXVIII) in crude form contamined with some of the BzMEA. Opening of I predominantly at C.3 is assumed on the basis of other experience with these blocked anhydromannosides.[1] Cleavage of XXXVIII with sodium in liquid ammonia afforded, in good yield, a crystalline thiol that was converted to the hydrochloride salt (XXXIX) without loss of the ethylidene blocking group. Treatment of XXXIX with methanolic hydrogen chloride gave the glycoside salt (XLI). Aqueous hydrolysis of XXXIX in an effort to prepare the free sugar coreesponding to XLI did not give a clean product. Aqueous acid hydrolysis of XXXIX could lead to 1,6-anhydride formation or to thioacetal formation by attack of the

[7] J. G. Moffatt and H. G. Khorana, J. Am. Chem. Soc. 83, 663 (1961)

pendant mercaptoethylamine group at C.1. Acetylation of the aqueous acid hydrolysate gave a sirup whose infrared spectrum showed no S-acetyl carbonyl absorption, suggesting that thioacetal formation had occurred.

Several attempts were made to prepare XLI by reaction of ethylene monothiolcarbonate[8] with a blocked derivative of methyl 3-amino-3-deoxy-α-D-altropyranoside, but little or no reaction could be realized, even in refluxing p-xylene.

When the anhydroalloside (XL) was treated with BzMEA, much more drastic conditions were required to cause complete reaction than in the case of I. No single product could be isolated from the reaction, possibly because of appreciable ring opening at C.3 as well as at C.2.[9]

D. Synthesis of 6-Amino-6-deoxy-L-idothiapyranose (XLV) Hydrochloride

Report 26 described the preparation of the crystalline amine (XLII) and its N-acetate (XLIII) that are possible precursors of the title compound (XLV) and preliminary attempts to convert XLII and XLIII to XLIV and XLVII, respectively. During this report period, the preparation of XLII has not been improved but further attempts to convert it to XLIV have not yet yielded material of satisfactory purity.

The cleavage of XLII with sodium and liquid ammonia followed by precipitation of the product with mercuric chloride gave a fair yield of a solid mercaptide, which analyzed well as the bis-mercurial (XLIX) (see Experimental). Decomposition of the mercaptide with hydrogen sulfide in methanol afforded a crystalline solid that gave a very faint nitroprusside test, as might be predicted for structure XLIV. The analytical data for this solid, however, were not satisfactory if structure XLIV is assumed. When the decomposition of the mercaptide was effected in dilute aqueous acid, the product again gave a very faint nitroprusside test but still failed to analyze well for structure XLV. These attempts to convert XLII to XLIV or XLV are continuing.

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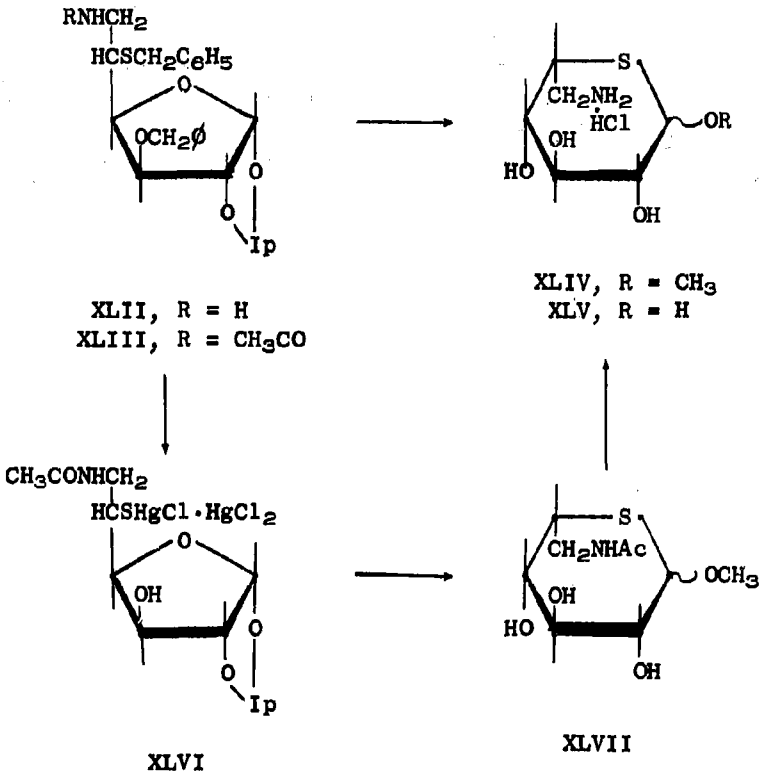
[8] D. D. Reynolds, M. K. Massad, D. L. Fields, and D. L. Johnson, J. Org. Chem. 26, 5109 (1961)

[9] See A. B. Foster, M. Stacey, and S. V. Vardheim, Acta Chem. Scand. 12, 1605 (1958), for the ammonolysis of such an anhydroalloside, and N. K. Richtmyer and C. S. Hudson, J. Am. Chem. Soc. 63, 1727 (1941), for the ring opening with potassium hydroxide.



As was described in Report 26, reduction of the N-acetate (XLIII) with sodium and liquid ammonia gave a thiol that formed a crystalline mercaptide whose elemental analyses were in good agreement with those of the trihydrate of XLVI. Hydrogen sulfide decomposition of XLVI gave a white solid that darkened on standing; this material may be the N-acetyl glycoside (XLVII); its analysis is in progress, as are attempts to convert it to XLV by acid hydrolysis.

IV Experimental[10]

Methyl-3-Benzylthio-3- deoxy-4,6-O-ethylidene-α-D-altropyranoside (II)

A solution of 8.00 g (39.6 mmoles) of I, 2.60 g (48.2 mmoles) of sodium methoxide, 6.03 g (48.5 mmoles) of benzylmercaptan, and 200 ml of methanol was stirred at reflux under nitrogen for 18 hours, cooled, and adjusted to pH 7 with glacial acetic acid, then poured into 400 ml of ice water. The product slowly solidified and was collected by filtration, washed with water and Skellysolve B, and dried, affording 12.53 g (97%) of solid. Recrystallization of the solid from 800 ml of Skellysolve C gave 11.41 g (89%) of product, mp. 132-133°. From a previous run an analytical sample was obtained with mp 132.0-132.5°; $\lambda_{max(\mu)}^{Nujol}$ 3.03 (OH), 14.00 (phenyl); $[\alpha]^{20}D$ -108°.

Anal. Calcd. for $C_{16}H_{22}O_5S$: C, 58.9; H, 6.80; S, 9.82. Found: C, 59.1; H, 6.51; S, 9.98.

Methyl 3(2)-Benzylthio-2(3)-chloro-2,3-dideoxy-4,6-O-ethylidene-α-D-altro(gluco)pyranoside (VI)

When 1.23 g (3.77 mmoles) of II was treated with 2.8 g (14.7 mmoles) of p-toluenesulfonyl chloride in 13 ml of dry pyridine, initially at 0° for 1 hour, then at room temperature for 48 hours, 0.85 g (ca. 65%) of sirupy product was isolated after a conventional work-up. The infrared spectrum showed no -OH absorption near 3.0 μ but did show some sulfonate ester absorption at 8.5 μ. Analysis verified the presence of some sulfonate ester.

Anal. Calcd. for $C_{16}H_{21}ClO_4S$: Cl, 10.28; S, 9.30. Found: Cl, 8.19; S, 9.98.

When the sulfonylation time was extended to 90 hours, an essentially quantitative yield of VI was isolated.

Anal. Calcd. for $C_{16}H_{21}ClO_4S$: C, 55.7; H, 6.14; Cl, 10.3; S, 9.30. Found: C, 56.8; H, 6.20; Cl, 10.6; S, 9.29.

Methyl 2-Azido-3-benzylthio-2,3-dideoxy-4,6-O-ethylidene-α-D-altropyranoside (V)

A stirred mixture of 11.9 g (34.5 mmoles) of crude VI, 27 g (0.42 mole) of sodium azide, and 300 ml of 95:5 2-methoxy-ethanol-water was heated at 100-110° under nitrogen for 18 hours, then evaporated in vacuo. The residue was partitioned between dichloromethane and water to yield, after drying, decolorizing with Norit A, and evaporating, 12.1 g of a partially crystalline sirup. Recrystallization of the crude product from 150 ml of Skellysolve C gave 7.09 g (58%) of crystalline solid, mp 137-138°. The analytical sample from another run had mp 137-138°; $\lambda_{max(\mu)}^{Nujol}$ 4.59, 4.70 and 4.78 (N_3); $[\alpha]^{28}D$ -99°.

[10]Boiling points and melting points are uncorrected; the latter were obtained with the Fisher-Johns apparatus. Optical rotations are given for 1% solutions in chloroform unless otherwise noted.

Anal. Calcd. for $C_{16}H_{21}N_3O_4S$: C, 54.7; H, 6.02; N, 12.0; S, 9.12. Found: C, 54.6; H, 6.09; N, 12.2; S, 9.30.

Methyl 2-Amino-3-mercapto-2,3-dideoxy-D-altropyranoside Hydrochloride (VII)

A solution of 2.00 g (5.7 mmoles) of the azide (V) in 12 ml of 1,2-dimethoxyethane was added dropwise, with stirring, to a solution of 0.80 g (0.0348 g-atom) of sodium in 35 ml of liquid ammonia. The resulting mixture was stirred at reflux, with exclusion of moisture, for 30 minutes, then the blue color was discharged with excess solid ammonium chloride. The ammonia was evaporated under a nitrogen atmosphere, the residue was dissolved in 10 ml of water, and the solution was adjusted to pH 7 with glacial acetic acid, then extracted with two 20-ml portions of dichloromethane while maintaining a nitrogen atmosphere. The combined extracts were washed with 17 ml of water, decolorized with Norit A, and dried over magnesium sulfate. Evaporation of the dried extract afforded 0.68 g of a yellow sirup whose infrared spectrum showed absorptions at 2.97 μ (NH_2) and 3.87 μ (SH). To the sirup was immediately added 3 ml of ethanedithiol followed by 20 ml of a 2% solution of hydrogen chloride in methanol, and the mixture was stirred at room temperature for 1 hour, then evaporated in vacuo to a semisolid residue that solidified when triturated with ether. The residue was reprecipitated from methanol ether to yield 0.61 g (44%) of cream-colored solid whose n.m.r. spectrum still showed some of the O-ethylidene methyl doublet centered at τ = 8.62. The solid was re-treated with methanolic hydrogen chloride and ethanedithiol at 50° for 5 hours, then worked up as before to give 0.49 g (33% as the etherate) of solid, which had a wide decomposition range and showed essentially no ethylidene methyl resonance in the n.m.r. spectrum but which did show the ether C-methyl triplet centered at τ = 8.78. The analytical sample was dried in vacuo at 100°. On paper chromatography[11] in system B it gave a major spot with R_{Ad} 1.31 with some material staying at the origin.

Anal. Calcd. for $C_7H_{16}ClNO_4S \cdot 1/8(C_2H_5)_2O$: C, 35.3; H, 6.82; N, 5.49; Cl, 13.9; S, 12.6. Found: C, 35.6; H, 6.82; N, 5.53; Cl, 13.8; S, 12.8.

When deblocking of IV was conducted in 2% methanolic hydrogen chloride at room temperature for 1 hour, the product (55% yield) was a hygroscopic solid, mp 124-146° dec; $\lambda_{max}^{Nujol}(\mu)$ 2.99 (OH weak), 4.92 and 6.25 ($NH_3^{\oplus}$); $[\alpha]^{27}D$ +27° (1% in methanol); it gave good elemental analyses for structure IX and its n.m.r. spectrum showed the prominent S,O-ethylidene methyl doublet centered at τ = 8.36.

Anal. Calcd. for $C_{18}H_{38}Cl_2N_2O_8S_2$: C, 38.5; H, 6.82; N, 4.99; Cl, 12.6; S, 11.4. Found: C, 38.6; H, 6.71; N, 5.06; Cl, 12.5; S, 11.4.

Methyl 2-Amino-3-benzylthio-2,3-dideoxy-4,6-O-ethylidene-α-D-altro pyranoside (VIII)

A mixture of 1.00 g (2.84 mmoles) of V, 0.25 (6.6 mmoles) of sodium borohydride, and 10 ml of isopropanol was stirred at reflux for 16 hours, then evaporated in vacuo. The residue was partitioned between dichloromethane and water, and the organic phase, after washing with water and drying, was evaporated in vacuo to afford 0.89 g (96%) of white solid. Recrystallization from Skellysolve C gave 0.67 g (72%) of the analytical sample, mp 123-124°; $\lambda_{max(\mu)}^{Nujol}$ 3.00 and 3.05 (NH_2), weak; $[\alpha]^{25}D$ -106°.

Anal. Calcd. for $C_{16}H_{23}NO_4S$: C, 59.1; H, 7.12; N, 4.30; S, 9.85. Found: C, 59.1; H, 7.24; N, 4.31; S, 9.93.

Methyl 3-Benzylthio-3-deoxy-α-D-arabinofuranoside (XII) and its Dibenzoate (XIII)

To a chilled (0°) solution containing 1.69 g (13.6 mmoles) of benzyl mercaptan, 0.74 g (13.7 mmoles) of sodium methoxide and 30 ml. of methanol was added 1.00 g (6.84 mmoles) of the epoxide (XII), and the resulting solution, under nitrogen, was heated at reflux for 18 hours. The mixture was adjusted to pH 7 with glacial acetic acid, then poured into 50 ml of water and extracted with two 25-ml portions of dichloromethane. The combined extracts were washed with 25 ml. of saturated aqueous sodium bicarbonate and two 25-ml portions of water, then dried over magnesium sulfate. The solvent was evaporated and the residue was washed by decantation with two 5-ml portions of Skellysolve B, then dried in vacuo to yield 1.73 g (94%) of a pale yellow sirup that was suitable for further use.

The dibenzoate (XIII) was prepared from 4.45 g (16.5 mmoles) of XII, 50 ml of pyridine, and 7.01 g (49.9 mmoles) of benzoyl chloride to afford, after decomposition of the reaction mixture, 6.70 g. (85%) of crude product. Recrystallization from 100 ml of Skellysolve C gave 5.51 g (70%) of product, m.p. 111-112°. The analytical sample, prepared in another experiment, had mp 110-111°; $\lambda_{max(\mu)}^{Nujol}$ 5.77, 5.81 (ester C=O), 7.79 (ester C-O-C), 14.05 and 14.26 (monosubstituted phenyl); $[\alpha]^{28}D$ +133°.

Anal. Calcd. for $C_{20}H_{22}O_5S$: C, 67.8; H, 5.48; S, 6.70. Found: C, 67.8; H, 5.90; S, 6.82.

A mixture of 25.9 g of the dibenzoate (XIII), 154 g of potassium hydroxide, 400 ml. of methanol, and 75 ml of water was heated at reflux for 4 hours, then cooled and evaporated in vacuo. The white residue was dissolved in 200 ml of water and the solution was extracted with one 200-ml and one 100-ml portion of dichloromethane. The combined extracts were washed with three 100-ml portions of water, dried over potassium carbonate, and evaporated in vacuo, yielding 10.2 g (70%) of pale yellow sirup, $N^{23}D$ 1.5580. The analytical sample, $N^{23}D$ 1.5560, $[\alpha]^{36}D$ +145°, was obtained similarly from another run after decolorizing the sirup with Norit A in benzene. In the infrared it had $\lambda_{max(\mu)}^{film}$ 2.91 (OH), 14.20 (monosubstituted phenyl).

Anal. Calcd. for $C_{13}H_{18}O_4S$: C, 57.8; H, 6.71, S, 11.9. Found: C, 58.0; H, 6.72; S, 11.7.

Methyl 3-Benzylthio-5-O-carbomethoxy-3-deoxy-α-D-arabinofuranoside (XIV)

To a chilled (0°) solution of 12.0 g (44.4 mmoles) of XII in 100 ml of dry pyridine was added dropwise, with stirring, a solution of 4.94 g. (52.2 mmoles) of methyl chloroformate in 50 ml. of chloroform. The mixture was stirred for one hour at 0° and at room temperature for 18 hours, then was poured, with stirring, into 400 ml of cold saturated aqueous sodium bicarbonate. The organic layer was separated and the aqueous layer was extracted with 100 ml of chloroform. The combined solutions were washed with 100 ml of water, dried over magnesium sulfate, and evaporated in vacuo. Pyridine was removed from the residue by the addition and evaporation of portions of toluene until the pyridine odor was gone. The residue, 14.5 g (100% yield), was a sirup, $\lambda_{max(\mu)}^{film}$ 2.90 (OH), 5.70 (ester C=O), 7.82 (ester C-O-C), 14.19 (monosubstituted phenyl).

Methyl 3-Benzylthio-5-O-carbomethoxy-3-deoxy-2-O-methylsulfonyl-α-D-arabinofuranoside (XVII)

To a chilled (0°), stirred mixture of 14.5 g (44.2 mmoles) of XIV in 100 ml of dry pyridine was added dropwise 13.4 g (0.116 mole) of methanesulfonyl chloride. The solution was stirred at 0° for 1 hour and at room temperature for 18 hours, then was poured into 500 ml of cold saturated aqueous sodium bicarbonate. The aqueous mixture was extracted with two 100-ml portions of dichloromethane; the combined extracts were washed with two 100-ml portions of water, then decolorized with Norit A and dried over magnesium sulfate. After removal of the solvent in vacuo, toluene was added and evaporated to remove all the pyridine, leaving 17.4 g (97%) of amber sirup, $\lambda_{max(\mu)}^{film}$ 5.70 (ester C=O), 7.33 and 8.50 (sulfonate ester), 7.83 (ester C-O-C), 14.15 (monosubstituted phenyl); there was no 3.0 μ -OH band.

Methyl 2(3)-Azido-3(2)-benzylthio-2,3-dideoxy-α-D-arabino(xylo)-furanoside (XVI)

A mixture of 17.4 g (42.8 mmoles) of XVII, 28 g (0.43 mole) of sodium azide, and 300 ml of 95:5 2-methoxyethanol-water was heated with stirring under nitrogen at 110-120° for 18 hours, then cooled and evaporated in vacuo. The residue was partitioned between 200 ml of water and 200 ml of dichloromethane. The aqueous layer was extracted with two 50-ml portions of dichloromethane; the combined extracts were washed with two 100-ml portions of water, then dried over magnesium sulfate. Evaporation in vacuo gave 11.9 g (94%) of a dark oil, $\lambda_{max(\mu)}^{film}$ 2.90 (OH), 4.77 (N_3), 14.22 (monosubstituted phenyl); there was no ester C=O band at 5.70 μ.

Anal. Calcd. for $C_{13}H_{17}N_3O_3S$: N, 14.2. Found: N, 14.9.

Methyl 2-Amino-3-benzylthio-2,3-dideoxy-α-D-arabinofuranoside Hydrochloride (XV) and Methyl 3-Amino-2-benzylthio-2,3-dideoxy-α-D-xylofuranoside Hydrochloride (XXII)

A mixture of 11.8 g (39.9 mmoles) of XVI, 3.5 g (93 mmoles) of sodium borohydride, and 150 ml of isopropanol was heated at reflux for 18 hours, then cooled and evaporated in vacuo. The residue was partitioned between 150 ml of water and 200 ml of dichloromethane, the resulting emulsion being broken after filtration through Celite. The dichloromethane extract was washed with 150 ml of water, dried over magnesium sulfate, and evaporated in vacuo, affording 10.2 g (95%) of orange sirup, $\lambda_{max(\mu)}^{film}$ 2.99, 3.04 (OH, NH_2); 6.25 (NH_2 and aryl), 14.20 (monosubstituted phenyl); the sirup was dissolved in 50 ml of cold (0°) pyridine containing 5.8 ml (42 mmoles) of triethylamine and the solution was treated with 25 ml of acetic anhydride. After it had stood at room temperature, the reaction mixture was added to 500 ml of ice water, the mixture neutralized with solid potassium carbonate and extracted with two 100-ml portions of dichloromethane. The combined extracts were washed with two 100-ml portions of water, decolorized with Norit A, and dried over magnesium sulfate. Evaporation in vacuo left 11.8 g (88%) of a foam that was a mixture of the amides (XIX and XX) which contained some of the diamides (XXIII and XXIV).

A mixture of the four amides (XIX, XX, XXIII and XXIV), prepared as above but containing more of the diamides (XXIII and XXIV), 32.0 g was applied to a column of Woelm neutral alumina, activity 1, 250 mm x 35 mm. Elution with 300 ml of ethyl acetate gave 18.7 g (A) which was largely XIX; a second 250 ml portion of ethyl acetate eluted 3.1 g (B) of almost pure XX, elution with 700 ml of chloroform yielded 4.0 g (C) of diamide which was largely XXII; and finally 200 ml more of chloroform eluted 0.50 g (D) of diamide which was mostly XXIV.

Fraction A, 18.7 g, was dissolved in a mixture of 250 g of potassium hydroxide, 170 ml of water, and 100 ml of methanol, and the solution was heated, under nitrogen, at reflux, for 40 hours, then evaporated in vacuo. The residue was dissolved in 200 ml of water and extracted with two 200-ml portions of dichloromethane, the extracts washed with water until neutral, then dried over magnesium sulfate and evaporated in vacuo, leaving 13.1 g (92%) of pale orange sirup which still contained a small amount of N-acetate according to the infrared spectrum. The residue was stirred with 60 ml of 5% methanolic hydrogen chloride for several minutes, then 100 ml of ether was added and the mixture was chilled. The white crystals, 2.16 g (14.4%), mp 211-213° dec, that separated were collected and shown to be the 2-amine salt (XXII) by infrared spectral comparison with the analytical sample. The filtrate from XXII, which had turned very dark, was evaporated in vacuo. Addition of 100 ml of cold acetonitrile yielded 4.21 g (28%) of a grey-brown solid whose infrared spectrum showed it to be XV, free from XXII. This was combined with 0.33 g of XV recovered from fraction D and the solid was recrystallized from 100 ml of acetonitrile to yield 2.70 g of needles of XV, mp 164-165°.

The saponification of fraction B, 3.1 g, gave 2.2 g (93%) of the amine mixture and this was converted to 0.86 g (34%) of XXII, mp 209-211° dec, and 0.33 g (13%) of XV, mp 150-159°, by the procedures described for fraction A.

From another run, 0.73 g of a pure mixture of XIX and XX was saponified using the general procedure described for conversion of fraction A (above), giving 0.44 g (79%) of the amine mixture. This was converted with 5 ml of 10% methanolic hydrogen chloride to 0.15 g (30%) of white needles, mp 209-210° dec, while the filtrate on evaporation and crystallization of the residue from acetonitrile afforded 0.16 g (32%) of white crystals, mp 158-162°.

Recrystallization of the higher melting salt (XXII) from methanol-ether gave the analytical sample, mp 210-212° dec; $[\alpha]^{25}D$ +59° (1% in methanol); $\lambda_{max(\mu)}^{Nujol}$ 2.99 (OH), 3.29, 3.66-3.82 (NH_3^+), 14.09 (monosubstituted phenyl).

Anal. Calcd. for $C_{13}H_{20}ClNO_3S$: C, 51.1; H, 6.59, Cl, 11.6; N, 4.58; S, 10.5. Found: C, 51.2; H, 6.71; Cl, 11.4; N, 4.53; S, 10.4.

Recrystallization of the lower melting salt (XV) from acetonitrile gave the analytical sample, mp 159-162°; $[\alpha]^{25}D$ +104° (1% in methanol); $\lambda_{max(\mu)}^{Nujol}$ 2.99 (OH); 3.21, 3.7-4.2 (NH_3^+), 13.95 (monosubstituted phenyl).

Anal. Calcd. for $C_{13}H_{20}ClNO_3S$: C, 51.1; H, 6.59; Cl, 11.6; N, 4.58; S, 10.5. Found: C, 51.3; H, 6.54; Cl, 11.6; N, 4.55; S, 10.5.

Methyl 2,5-Acetamido-3-benzylthio-2,3,5-trideoxy-α-D-arabinofuranoside (XXIII)

Fraction C, 4.0 g, from the chromatographic separation of the amide mixture (see above experiment) was recrystallized from ethyl acetate, giving 2.75 g of solid, mp 162-170°, then again from ethyl acetate, giving 1.54 g of crystals, mp 163-166°. A third recrystallization from ethyl acetate gave 0.84 g of the analytical sample, mp 164-166°, $[\alpha]^{24}D$ +43.7°; $\lambda_{max(\mu)}^{Nujol}$ 3.02, 3.03; 6.40 (NH); 6.01, 6.03 (amide C=O); 13.07, 14.03 (monosubstituted phenyl. The n.m.r. spectrum, run in deuterochloroform, showed resonances at τ = 2.70 (aromatic); 4 (broad, NH); 5.12 (C.1, essentially single peak or doublet with J ~ 1.5 cps); 6.18 (benzyl CH_2); 6.66 (OCH_3); 7.99 and 8.12 (CH_3CO).

Anal. Calcd. for $C_{17}H_{24}N_2O_4S$: C, 57.9; H, 6.86; N, 7.95; S, 9.10. Found: C, 57.9; H, 6.82; N, 7.98; S, 9.09.

Methyl 3,5-Acetamido-2-benzylthio-2,3,5-trideoxy-α-D-xylofuranoside-(XXIV)

Fraction D, 0.50 g, from the chromatographic separation of the amide mixture was recrystallized from ethyl acetate affording 0.10 g of solid,

mp 231-235°; $[\alpha]^{24}D$ -19.6; $\lambda_{max(\mu)}^{Nujol}$ 3.01, 3.03, 6.40 (NH), 6.03 (amide C=O), 13.02, 14.27 (monosubstituted phenyl). The n.m.r. spectrum, in deuterochloroform, showed resonances at τ = 2.68 (aromatic), 4 (broad, NH), 5.22 (C.1, doublet, J ~4.5 cps), 6.23 (benzyl CH_2), 6.65 (OCH_3), 7.98 and 8.10 (CH_3CO).

Anal. Calcd. for $C_{17}H_{24}N_2O_4S$: C, 57.9; H, 6.86; N, 7.95; S, 9.10. Found: C, 58.0; H, 6.98; N, 7.96; S, 9.01.

Methyl 2-Acetamido-5-O-acetyl-3-benzylthio-2,3 dideoxy-α-D-arabino-furanoside (XIX)

A mixture of 0.35 g (1.14 mmoles) of XV, 4 ml of dry pyridine, 0.30 ml of triethylamine and 5 ml of acetic anhydride was maintained at room temperature for 18 hours then poured into ice water. The solid was collected and dried yielding 0.28 g (69%) of white crystals. Recrystallization from water afforded 0.26 g (64%) of needles, mp 134-136°; $[\alpha]^{24}D$ +194°; $\lambda_{max(\mu)}^{Nujol}$ 3.11 and 6.40 (NH), 5.75 (ester C=O), 6.08 (amide C=O), 8.07 (ester C-O-C), 14.13 (monosubstituted phenyl). The n.m.r. spectrum, in deuterochloroform, showed resonances at τ = 2.70 (aromatic), 4-5 (broad, NH), 5.18 (C.1, singlet); 6.16 (benzyl CH_2), 6.64 (OCH_3); 7.96 and 8.06 (CH_3CO).

Anal. Calcd. for $C_{17}H_{23}NO_5S$: C, 57.8; H, 6.56; N, 3.96; S, 9.07. Found: C, 57.9; H, 6.62; N, 3.96; S, 9.21.

Methyl 3-Acetamido-5-O-acetyl-2-benzylthio-2,3-dideoxy-α-D-xylofurano-side (XX)

Acetylation of 0.35 g (1.14 mmoles) of XXII by the procedure described for preparation of XIX gave after recrystallization from water 0.37 g (92%) of white crystals, mp 138-139° (mixed melting point with XIX was 113-116° $[\alpha]^{24}D$ +133°; $\lambda_{max(\mu)}^{Nujol}$ 3.09 and 6.62 (NH), 5.74 (ester C=O), 6.05 (amide C=O), 8.14 (ester C-O-C), 14.40 (monosubstituted phenyl). The n.m.r. spectrum, in carbon tetrachloride, showed resonances at τ = 2.66 (aromatic); 4-5 (broad, NH); 5.16 (C.1, doublet, J ~4.5 cps); 6.21 (benzyl CH_2); 6.63 (OCH_3); 7.98 and 8.11 (CH_3CO).

Anal. Calcd. for $C_{17}H_{23}NO_5S$: C, 57.8; H, 6.56; N, 3.96; S, 9.07. Found: C, 58.1, H, 6.61; N, 3.94, S, 9.26.

Methyl 2-Amino-2,3-dideoxy-3-mercapto-α-D-arabinofuranoside Hydro-chloride (XVIII)

The free base of XV was generated by dissolving 1.45 g (4.73 mmoles) of XV in 30 ml of saturated aqueous sodium bicarbonate. The solution was extracted with three 15-ml portions of dichloromethane; the extracts were washed with water, dried over magnesium sulfate, and evaporated in vacuo, giving 1.23 g (96%) of the free amine. A solution of the amine in 8 ml of dry 1,2-dimethoxyethane was added dropwise to a well stirred solution

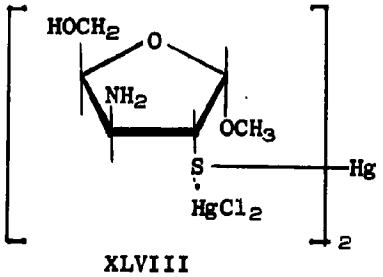
of 0.55 g (24 mg atoms) of sodium in 25 ml of liquid ammonia. The mixture was stirred for 30 minutes, then the excess sodium was decomposed by adding absolute ethanol to discharge the blue color. The ammonia was evaporated under nitrogen and the residue was dissolved in 8 ml of water. The aqueous solution was adjusted to pH 7 with glacial acetic acid and was then treated with excess aqueous mercuric chloride solution. The white precipitate was washed with water and dried, then thoroughly triturated with dichloromethane to remove bibenzyl, affording finally 2.51 g of white solid mercaptide.

The mercaptide was suspended in 25 ml of methanol and hydrogen sulfide was bubbled through the well stirred suspension for 20 minutes. The reaction mixture was filtered through Celite and the filtrate was evaporated in vacuo, affording 0.86 g (84%) of a tan, crystalline residue. The residue was dissolved in 15 ml of methanol and precipitated with excess ether, giving a gummy product that solidified to a foam after being treated in vacuo. The nitroprusside-positive solid, 0.59 g (58%), had $[\alpha]^{24}D$ +100.8° (1% in methanol, $\lambda_{max(\mu)}^{Nujol}$ 2.87, 2.96 (OH), 3.82 (SH), 5.0, 6.22 and 6.55 ($NH_3^{\oplus}$); there was no phenyl absorption near 14 μ. It was homogeneous on paper chromatography[11] in solvents A and B with R_{Ad} 0.96 and 1.38, respectively.

Anal. Calcd. for $C_6H_{14}ClNO_3S$: C, 33.4; H, 6.54; Cl, 16.4; N, 6.49; S, 14.9. Found: C, 33.4; H, 6.63; Cl, 16.7; N, 6.31; S, 14.8.

Methyl 3-Amino-2,3-dideoxy-2-mercapto-α-D-xylofuranoside Hydrochloride (XXI)

The salt (XXII), 2.40 g (7.83 mmoles), was converted to its free base and thence to its mercaptide using the procedure described for the similar conversion of XV. The mercaptide was a brown solid, 4.81 g (112%). In a previous run the mercaptide had been analyzed and appeared to be the bismercury compound (XLVIII).



XLVIII

[11] Paper chromatography was run by the descending technique on Whatman No. 1 paper, using solvent systems A, n-butanol-acetic acid-water (5:2:3) and B, isopropyl alcohol-2N hydrochloric acid (65:35). Spots were detected with the sodium azide-iodine spray,[12] unless otherwise noted and were located relative to adenine, (R_f adenine = 1.00).

[12] E. Chargoff, C. Levine and C. Green, Biol. Chem., 175, 67 (1948).

Anal. Calcd. for $C_{12}H_{24}Cl_4Hg_3N_2O_6S_2$: C, 13.1; H, 2.20, Cl, 12.9, N, 2.55. Found: C, 12.7; H, 1.84; Cl, 12.9; N, 2.51.

The mercaptan was generated from XLVIII with hydrogen sulfide and gave 1.00 g (59%) of crystalline solid, which was reprecipitated from methanol with ether to give 0.73 g (43%) of the nitroprusside positive analytical sample, $[\alpha]^{24}D$ +73.5° (1% in methanol); $\lambda_{max(\mu)}^{Nujol}$ 3.02, 3.17 (OH), 3.83 (SH), 6.22, 6.40, 6.60 ($NH_3^{\oplus}$); there was weak and unexpected absorption at 14.3 μ. The product was homogeneous on paper chromatography[11] in solvents A and B with R_{Ad} 0.98 and 1.36, respectively.

Anal. Calcd. for $C_8H_{14}ClNO_3S$: C, 33.4; H, 6.54, Cl, 16.4; N, 6.49; S, 14.9. Found: C, 33.5; H, 6.78; Cl, 16.7; N, 6.73; S, 14.6.

Methyl 3-Deoxy-3-(dithiocarbomethoxy)amino-α-D-arabinofuranoside (XXVI) and its Dibenzoate (XXVII).

To a chilled (0°), stirred solution of 22.3 g (0.137 mole) of the amine (XXV), 400 ml of dry pyridine, and 14.3 g (0.141 mole) of dry triethylamine was added slowly, while maintaining the temperature below 10°, 11.2 g (0.146 mole) of carbon disulfide. The solution was stirred for one hour at 0-5°, then 20.1 g (0.141 mole) of iodomethane was added, slowly while the temperature was kept below 10°. The mixture was stored at 5° for 24 hours, then was poured with stirring into 1 liter of ice water. The product was extracted with two 250-ml portions of dichloromethane, the combined extracts were washed with water, dried over magnesium sulfate, and evaporated in vacuo, affording, after removal of all the pyridine, 32.6 g (94%) of a yellow solid. Recrystallization of the solid from 100 ml of benzene gave 26.7 g (77%) of crystals, mp 85-90°, and a second recrystallization from 85 ml of benzene yielded 25.2 g (73%) of product, mp 88-91°. The analytical sample, recrystallized from benzene, had mp 91-92°, $[\alpha]^{28}D$ +92° (1% in methanol); $\lambda_{max(\mu)}^{Nujol}$ 2.99, 3.01, 3.05 (OH, NH), 6.63 (NH).

Anal. Calcd. for $C_8H_{15}NO_4S_2$: C, 37.9; H, 5.97; N, 5.53; S, 25.3. Found: C, 37.9; H, 5.69; N, 5.31; S, 25.5.

A mixture of 0.97 g (3.83 mmoles) of XXVI and 10 ml of pyridine was treated with 1.70 (12.0 mmoles) of benzoyl chloride according to standard procedures. The crude yield of solid was 1.72 g (97%) and this was recrystallized from 15 ml of benzene and sufficient petroleum ether (30-60°) to cause crystallization, affording 1.41 g (80%) of plates, mp 118-125°. The analytical sample recrystallized from benzene had mp 125-127°; $[\alpha]^{24}D$ -33.0°; $\lambda_{max(\mu)}^{Nujol}$ 3.03, 6.53 (NH); 5.78, 5.83 (ester C=O); 7.79 (ester C-O-C), 13.90 (monosubstituted phenyl).

Anal. Calcd. for $C_{22}H_{23}NO_6S_2$: C, 57.3; H, 5.02; N, 3.04; S, 13.9. Found: C, 58.0; H, 5.28, N, 2.91; S, 13.6.

Methyl 5-O-Carbomethoxy-3-deoxy-3-(dithiocarbomethyl)amino-α-D-arabinofuranoside (XXX)

To a chilled (0°), stirred solution of 13.36 g (52.7 mmoles) of XXVI in 80 ml of pyridine was added dropwise a solution of 5.42 g (57.6 mmoles) of methyl chloroformate in 40 ml of chloroform. The resulting solution was stirred for 30 minutes at 0° and for 18 hours at room temperature, then poured, with stirring, into 500 ml of cold saturated aqueous sodium bicarbonate. The chloroform layer was separated and the aqueous layer was extracted with 100 ml of chloroform. The combined chloroform solutions were washed with two 100-ml portions of water, dried over magnesium sulfate, and evaporated in vacuo, finally adding and evaporating toluene to remove most of the pyridine, giving 18.7 g of yellow sirup that still contained some pyridine; $\lambda_{max(\mu)}^{film}$ 2.90-3.02 (OH, NH), 5.68 (ester C=O), 6.62 (NH), 7.82 (C-O-C).

Methyl 5-O-Carbomethoxy-3-deoxy-3-(dithiocarbomethoxy)amine-2-O-methylsulfonyl-α-D-arabinofuranoside (XXIX)

The crude XXX from the preceding experiment, dissolved in 200 ml of dry pyridine and chilled in an ice acetone bath, was treated with 12.9 g (0.148 moles) of methanesulfonyl chloride, added dropwise and with good stirring. The solution was stirred one hour in the ice-acetone bath and was stored at 5° for 18 hours, then was poured with stirring into 500 ml of cold saturated aqueous sodium bicarbonate. The product was extracted with two 100-ml portions of dichloromethane and the extracts were washed with two-100-ml portions of water, dried over magnesium sulfate and evaporated in vacuo at room temperature. Two 50-ml portions of toluene were added and evaporated to remove pyridine and 50 ml of toluene was added to the solid residue. Filtration of the mixture gave 10.92 g (53%) of solid, 94-110°. The filtrate was evaporated in vacuo and ethanol was added to the residue, affording an additional 1.12 g (5.5%) of product, mp 113-120°. From a previous run the analytical sample was obtained after several recrystallizations from ethyl acetate, mp 123-125°; $[\alpha]^{26}D$ +116°; $\lambda_{max(\mu)}^{Nujol}$ 3.06, 6.59 (NH), 5.80 (ester C=O), 7.72 (ester C-O-C), 7.40 and 8.50 (sulfonate ester).

Anal. Calcd. for $C_{11}H_{19}NO_8S_3$: C, 33.9; H, 4.92; N, 3.60; S, 24.7. Found: C, 33.9; H, 4.85; N, 3.34; S, 23.2, 23.5.

5'-O-carbomethoxy-1'-O-methyl-2-(methylthio)-α-D-ribofurano-[3',2':4,5]-2-thiazoline (XXVIII)

A From hot pyridine

A solution of 6.06 g (15.6 mmoles) of XXIX in 60 ml of pyridine was heated at reflux for 3-1/2 hours under a nitrogen atmosphere, then cooled and evaporated in vacuo. The residue was extracted with four 30-ml portions of hot benzene leaving a residue of pyridinium mesylate. The residues from two identical runs were combined, decolorized with Norit A, dried over magnesium sulfate and evaporated in vacuo leaving 8.92 g (98%) of an amber sirup; $\lambda_{max(\mu)}^{film}$ 5.70 (ester C=O), 6.37 (C=N), 7.85 (ester C-O-C).

B From hot toluene

A solution of 2.00 g (5.14 mmoles) of XXIX in 50 ml of toluene was heated at reflux for 75 minutes, during which time an oil separated. The mixture was chilled and the toluene decanted. The residue was washed twice by decantation with ether and dried, yielding 1.28 g (64%) of an amber sirup which appears to be the mesylate salt of XXVIII; $\lambda^{film}_{max(\mu)}$ 3.70 ($NH^{\oplus}$), 5.69 (ester C=O), 6.40 (C=N), 7.82 (ester C-O C), 8.38, 9.41, 9.61 (mesylate ion). The salt, treated with sodium methoxide, afforded 0.54 g. (70%) of XXXI; aluminum amalgam reduction of XXXI gave 0.48 g (110%) of crude XXXII. The thiazolidine XXXII was converted to 0.98 g of mercaptide and thence to 0.16 g (55%) of analytically pure mercaptoamine salt (XXXIII).

1'-O-methyl-2-(methylthio)-α-D-ribofurano-[3',2':4,5]-2-thiazoline (XXXI)

A mixture of 8.92 g (30.5 mmoles) of XXVIII, 150 ml of methanol, and 0.3 g of sodium methoxide was stirred at room temperature overnight, adjusted to pH 7 with glacial acetic acid, and evaporated in vacuo. The residue was partitioned between 100 ml of dichloromethane and 50 ml of water and the organic layer was washed with 50 ml of water, then dried over magnesium sulfate. Evaporation gave 5.69 g (79%) of a sirup; $\lambda^{film}_{max(\mu)}$ 2.96 (OH), 6.38 (C=N).

1'-O-Methyl-α-D-ribofurano-[3',2':4,5]-thiazolidine (XXXII)

Aluminum foil (14 g) was amalgamated according to the directions of Vogel[13] and to the aluminum amalgam was added a solution of 5.69 g (24.1 mmoles) of XXXI in 500 ml of tetrahydrofuran. The well stirred mixture was chilled and 50 ml of water was added, dropwise with stirring. The reaction mixture was stirred for 6 hours at 55-60°, then cooled, filtered through Celite, and evaporated in vacuo to afford 4.2 g (91%) of a sirup; $\lambda^{film}_{max(\mu)}$ 2.93-3.05 (OH, NH); there was no C=N absorption near 6.4 μ.

Methyl 3-Amino-2,3-dideoxy-2-mercapto-α-D-ribofuranoside Hydrochloride (XXXIII)

A solution of 2.00 g (10.5 mmoles) of XXXII in 10 ml of water was filtered through Celite to remove a small amount of insoluble material, then was treated with excess aqueous mercuric chloride solution to give, after drying, 5.33 g of a cream-colored solid. The solid was suspended in 50 ml of methanol and hydrogen sulfide was bubbled through the well stirred suspension for 20 minutes. The mixture was filtered through Celite and the filtrate evaporated in vacuo. The residue was washed with ether and dried in vacuo, leaving 1.36 g (85%) of a white, nitroprusside-positive foam; $\lambda^{Nujol}_{max(\mu)}$ 3.0-3.2 (OH), 5.0, 6.29, 6.69 ($NH_3^{\oplus}$).

Anal. Calcd. for $C_6H_{14}ClNO_3S$: C, 33.4; H, 6.54; Cl, 16.4; N, 6.49; S, 14.9. Found: C, 33.2; H, 6.61; Cl, 16.6; N, 6.40; S, 14.9.

[13] A.I. Vogel, "Textbook of Practical Organic Chemistry," Longmans, Green and Co., Ltd., London, Eng., 1956, p. 198

A previous analytical sample (A) had $[\alpha]^{24}D$ -78° (1% in methanol); on paper chromatography[11] in solvent A it showed two spots with R_{Ad} 0.41 and 0.97. Another sample (B) of analytical purity had $[\alpha]^{28}D$ -61° (1% in methanol), suggesting that different samples may have been subjected to different degrees of anomerization during preparation.

Anal. Found: (for A): C, 33.2; H, 6.55; Cl, 16.3; N, 6.30; S, 15.0. (for B) C, 33.4; H, 6.77; Cl, 16.2; N, 6.36; S, 14.6.

Methyl 2,5-Di-O-methylsulfonyl-3-(dithiocarbomethoxy)amino-α-D-arabinofuranoside (XXXV)

A chilled (0°), stirred mixture of 5.00 g (19.8 mmoles) of (XXVI) in 80 ml of pyridine was treated dropwise with 7.4 g (64.7 mmoles) of methanesulfonyl chloride under standard sulfonylation conditions. After a standard workup, utilizing extraction with dichloromethane, there was obtained 7.62 g (94%) of a sirup; $\lambda_{max(\mu)}^{film}$ 3.03, 6.55 (NH); 7.35, 8.50 (sulfonate ester).

1'-O-Methyl-2'-O-methylsulfonyl-2-(methylthio)-α-D-arabinofurano-[5',3':4,5]-4,5-dihydro-6H-1,3-thiazine (XXXIV)

A. From XXIX

A mixture of 1.00 g (2.57 mmole) of XXIX in 10 ml of methanol was treated with a solution of 0.150 g (2.78 mmoles) of sodium methoxide in 10 ml of methanol. The solution was stirred at room temperature for 5 minutes and heated on the steam bath for 3 minutes, then chilled and treated with a large volume of water. The precipitate, 0.19 g (24%), mp 101-103°, was recrystallized twice from Skellysolve C to give 0.13 g (16%) of crystals, mp 111-113°; $[\alpha]^{24}D$ -93.9°; $\lambda_{max(\mu)}^{Nujol}$ 6.35 (C=N), 7.39, 8.43 (sulfonate ester); there was no OH absorption near 3.0 μ.

Anal. Calcd. for $C_9H_{15}NO_5S_3$: C, 34.5; H, 4.82; N, 4.47; S, 30.7. Found: C, 34.9; H, 4.98; N, 4.23; S, 31.0.

B. From XXXV

To a stirred, chilled (-11°) solution of 4.30 g (10.5 mmoles) of the dimesylate (XXXV) in 20 ml of methanol was added a solution of 0.60 g (11.1 mmoles) of sodium methoxide in 20 ml of methanol. The solution was stirred for 10 minutes then poured into about 100 ml of water. The precipitate, 2.08 g (64%), was recrystallized from Skellysolve C to give 1.32 g (40%) of white crystals. A second recrystallization from Skellysolve C gave product with mp 110-112°.

1'-O-methyl-2'-O-methylsulfonyl-α-D-arabinofurano-[5',3':4,5]-tetrahydro-1,3-thiazine (XXXVI)

A chilled, stirred suspension of 3.0 g of aluminum amalgam B, 1.20 g (3.84 mmoles) of XXXV, and 100 ml of tetrahydrofuran was treated, dropwise, with 15 ml of water. The mixture was heated with stirring at 50° for 18 hours under a nitrogen atmosphere, then was cooled and filtered through Celite. The filtrate was evaporated in vacuo, the residue partitioned between 20 ml of dichloromethane and 20 ml of water, and the extract washed with 10 ml of water, then dried over magnesium sulfate. Evaporation in vacuo left 1.20 g (116%) of a sirup which crystallized when it was scratched. This was recrystallized from ethanol, yielding 0.45 g (44%) of crystals, mp 122-125°. The analytical sample from ethanol had mp 125-127°, $[\alpha]^{25}D$ +80°; $\lambda_{max(\mu)}^{Nujol}$ 3.07 (NH), 7.37, 8.50 (sulfonate ester); there was no C=N absorption near 6.4 μ.

Anal. Calcd. for $C_8H_{15}NO_5S_2$: C, 35.7; H, 5.61; N, 5.20; S, 23.8. Found: C, 35.6; H, 5.69; N, 5.22; S, 23.9.

Methyl 3-Amino-3,5-dideoxy-5-mercapto-2-O-methylsulfonyl-α-D-arabinofuranoside Hydrochloride (XXXVII)

The tetrahydro-1,3-thiazine (XXXVI), 0.30 g (1.1 mmoles), was dissolved in 10 ml of boiling water and to the hot solution was added an excess of saturated aqueous mercuric chloride. The white precipitate, 0.82 g, was collected and suspended in 10 ml of methanol. Hydrogen sulfide was bubbled through the well stirred suspension for 15-20 minutes, then the mixture was filtered through Celite and the filtrate evporated in vacuo. The residue was washed with ether and dried in vacuo to afford 0.23 g of a white, nitroprusside-positive foam; $\lambda_{max(\mu)}^{Nujol}$ 2.95 (OH), 4.85-5.0, 6.25, 6.69 ($NH_3^{\oplus}$), 7.32, 8.47 (sulfonate ester).

Anal. Calcd. for $C_7H_{16}ClNO_5S_2$: C, 28.6; H, 5.49; Cl, 12.1; N, 4.77; S, 21.3. Found: C, 29.7; H, 5.64; Cl, 11.7; N, 4.68; S, 21.2.

Methyl 3-(β-Benzylthioethyl)amino-3-deoxy-4,6-O-ethylidene-α-D-altropyranoside (XXXVIII)

A mixture of 12.0 g (59.0 mmoles) of S-benzyl-β-mercaptoethylamine hydrochloride[7] in 120 ml of 1 M aqueous sodium hydroxide was stirred for 10 minutes, then extracted with two 80-ml portions of dichloromethane. The combined extracts were washed with 80 ml of water, dried over potassium carbonate, filtered, and evaporated in vacuo to afford 9.86 g (59.0 mmoles) of S-benzyl-β-mercaptoethylamine as a sirup, which was added to 40 ml of dimethyl sulfoxide. This solution was added to a solution of 4.00 g (19.8 mmoles) of the anhydromannoside (I) in 40 ml of dimethyl sulfoxide and the reaction mixture was stirred at 110-115° for 18 hours in a nitrogen atmosphere, then poured, with stirring into 500 ml of ice water. The mixture was extracted with three 100-ml portions of chloroform, the combined extracts were washed with two 100-ml portions of water, dried over potassium carbonate, and evaporated in vacuo, giving a yellow liquid.

The residue was triturated with five 30-ml portions of Skellysolve B, decanting each time, affording finally 5.48 g (75%) of a yellow sirup whose nitrogen analysis suggested that it contained about 10% of S-benzyl-β-mercaptoethylamine.

Methyl 3-(β-Mercaptoethyl)amino-3-deoxy-4,6-O-ethylidene-α-D-altropyranoside Hydrochloride (XXXIX)

A solution of 2.89 g (7.05 mmoles, calculated as 90% pure) of crude XXXVIII in 17 ml of 1,2-dimethoxyethane was added dropwise and with stirring to a solution of 0.90 g (0.039 g-atom) of sodium in 40 ml of liquid ammonia. The mixture was stirred for 30 minutes under reflux, then the blue color was discharged by the addition of excess solid ammonium chloride. The ammonia was evaporated under nitrogen, 30 ml of water was added to the residue, and the aqueous solution was adjusted to pH 7 with glacial acetic acid. The aqueous solution was extracted with three 20-ml portions of dichloromethane, the combined extracts were washed with 10 ml of water, dried over magnesium sulfate while maintaining a nitrogen atmosphere, then filtered and evaporated in vacuo to give 1.58 g (80%) of a pale yellow, crystalline solid whose infrared spectrum showed an -SH absorption band at 3.9 μ.

The solid was immediately dissolved in 10 ml of 1,2-dimethoxyethane and to this solution was added, with cooling and stirring, 20 g of a 2.2% solution of hydrogen chloride in 1,2-dimethoxyethane. The crystalline solid, 1.44 g (64%), mp 183-191° dec, was collected and recrystallized by dissolving in warm methanol, then adding enough ether to initiate crystallization. The analytical sample had mp 178-186° dec; $\lambda_{max(\mu)}^{Nujol}$ 3.00 (OH), 3.59, 3.69, 3.72, 6.27 ($NH_2^{\oplus}$); $[\alpha]^{30}D$ +89° (1% in methanol); it traveled as a single spot with R_{Ad} 1.16 on paper chromatography in solvent A with detection by sodium nitroprusside.

Anal. Calcd. for $C_{11}H_{22}ClNO_5S$: C, 41.8; H, 7.02; Cl, 11.2; N, 4.44; S, 10.2. Found: C, 41.9; H, 6.64; Cl, 11.2; N, 4.41; S, 10.3.

Some anomerization evidently accompanied the recrystallization, since another sample taken directly from the hydrogen chloride treatment had mp 184-190° dec and $[\alpha]^{27}D$ +96° (1% in methanol).

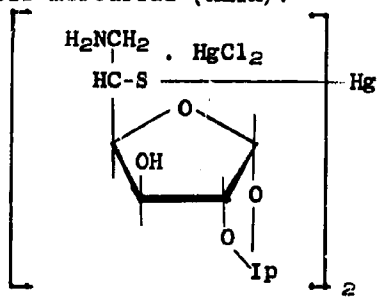
Methyl 3-(β-Mercaptoethyl)amino-3-deoxy-D-altropyranoside Hydrochloride (XLI)

A solution of 5.0 g of XXXIX in 300 ml of 1% hydrogen chloride in methanol was heated at reflux for 3 hours, then evaporated in vacuo. The residue was triturated several times with ether and dried, yielding 4.23 g (92%) of a yellow, hygroscopic, nitroprusside-positive solid, mp 50-70°; $\lambda_{max(\mu)}^{Nujol}$ 3.05 (OH), 6.32 ($NH_2^{\oplus}$); it moved as a single spot on paper chromatography in solvent A with R_{Ad} 0.70, with detection by sodium nitroprusside.

Anal. Calcd. for $C_9H_{20}ClNO_5S$: C, 37.3; H, 6.96; Cl, 12.2; N, 4.83; S, 11.1. Found: C, 37.0; H, 6.98; Cl, 13.0, 12.8; N, 4.73, S, 11.4.

Methyl 6-Amino-6-deoxy-L-idothiapyranoside Hydrochloride (XLIV)

A solution of 1.50 g (3.60 mmoles) the amine (XLII) in 5 ml of 1,2-dimethoxyethane was added dropwise, with stirring, to a solution of 0.40 g (17 mg-atoms) of sodium in 20 ml of liquid ammonia. The solution was stirred 30 minutes, the excess sodium was destroyed with solid ammonium chloride, and the ammonia was allowed to evaporate under a nitrogen atmosphere. The residue was dissolved in 50 ml of water and neutralized with glacial acetic acid. The addition of excess saturated aqueous mercuric chloride yielded a precipitate which was dried and triturated throughly with dichloromethane to remove bibenzyl. The mercaptides, 1.93 g, analyzed well as the bis-mercurial (XLIX).



XLIX

Anal. Calcd. for $C_{18}H_{32}Cl_4Hg_3N_2O_8S_2$: C, 17.8; H, 2.66; Cl, 11.7; N, 2.31; S, 5.29. Found: C, 17.5; H, 3.03; Cl, 11.6; N, 2.09; S, 5.09.

The mercaptide (XLIX), 1.80 g (2.31 mmoles), was suspended in 15 ml methanol and the well stirred suspension was treated with hydrogen sulfide for 20 minutes, then filtered through Celite. The filtrate was evaporated in vacuo, yielding 0.66 g of a solid foam whose infrared spectrum showed that much of the isopropylidene group was still present. The solid was precipitated from a methanol solution with ether, affording 0.50 g (88%) of a white solid that gave a very weak nitroprusside test. A portion of the solid, 100 mg, was stirred for 2-1/2 hours with 10 ml of 1% methanolic hydrogen chloride to give, after evaporation in vacuo, 60 mg of an orange foam that was reprecipitated with ether from a methanol solution, yielding 40 mg of a cream colored solid that gave a very weak nitroprusside test and that showed no infrared isopropylidene absorptions.

Anal. Calcd. for $C_7H_{16}ClNO_4S$: C, 34.2; H, 6.56; Cl, 14.4; N, 5.70; S, 13.1. Found: C, 32.2; H, 6.34; Cl, 15.5; N, 5.75; S, 13.7.

The remainder of the original solid, 0.40 g, was similarly treated with 1% methanolic hydrogen chloride and the product was recrystallized from methanol, affording a solid, mp 151-154° dec; $[\alpha]^{25}D$ +9.0° (0.5% in water); $\lambda_{max(\mu)}^{Nujol}$ 2.97-3.2 (OH, $NH_3^{\oplus}$), 5.0, 6.23, 6.64 ($NH_3^{\oplus}$).

Anal. Found: C, 32.2; H, 6.31; Cl, 15.2; N, 6.02.

Another portion of the mercaptide (XLIX), dissolved in methanol, was treated with hydrogen sulfide as above except that the reaction mixture was stirred for 4 hours in order to complete the removal of the isopropylidene. The product obtained in 77% yield after reprecipitation from methanol, was a foam.

Anal. Found: C, 30.2; H, 6.01; Cl, 14.5; N, 5.56; S, 12.9.

6-Amino-6-deoxy-L-idothiapyranose Hydrochloride (XLV)

The mercaptide (XLIX), 3.60 g (2.97 mmoles) was suspended in 20 ml of water that contained 1.2 ml (14.4 mmoles) of concentrated hydrochloric acid. The well stirred suspension was treated with hydrogen sulfide for 20 minutes, then filtered through Celite, and evaporated in vacuo as in the case of XLIV. The crude product, 1.06 g, which gave a positive Benedict's test, was dissolved in 10 ml of methanol, the solution was decolorized with Norit A and the product precipitated with ether affording 0.72 g (104%) of a white solid that gave a very weak nitroprusside test and gave a positive Benedict's test, mp 60-90° dec. On paper chromatography[11] in solvent A, the material showed three spots when detected with either sodium azide-iodine[12] or with aniline citrate.

Anal. Calcd. for $C_8H_{14}ClNO_4S$: C, 31.1; H, 6.08; Cl, 15.3; N, 6.05; S, 13.8. Found: C, 30.0; H, 6.05; Cl, 19.2; N, 6.91; S, 12.9.

Methyl 6-Acetamido-6-deoxy-L-idothiapyranoside (XLVII)

A suspension of 1.24 g (1.58 mmoles) of the mercaptide (XLVI), mp 125-128°, in 15 ml of methanol was treated with hydrogen sulfide using the procedure for the preparation of LIV. A portion of the crude product, 0.44 g (110%), was precipitated from a methanol solution, which had been decolorized with Norit A, with ether, affording a hygroscopic foam.

Anal. Calcd. for $C_8H_{17}NO_5S$: C, 43.0; H, 6.82; N, 5.57. Found: C, 39.9; H, 6.48; N, 5.19 (the C:H:N ratio is 9.0:17.4:1.0 as suggested by the empirical formula).

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